Original Research Paper

Volume - 16 | Issue - 07 | July - 2026 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Medical Microbiology

BIOFILM PRODUCTION BY UROPATHOGENS AND ANTIBIOTIC RESISTANCE AT SHADAN HOSPITAL, HYDERABAD, TELANGANA.

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ABSTRACT **Background:** Urinary tract infections remain among the most common bacterial infections encountered in hospitalized patients, particularly in those with prolonged catheterization. The emergence of biofilm-producing uropathogens has become a major clinical concern because biofilms enhance bacterial adherence, persistence, and resistance to antimicrobial agents. Biofilm-associated infections are difficult to eradicate and are frequently associated with recurrent infections, prolonged hospital stay, increased healthcare costs, and therapeutic failure. The increasing prevalence of multidrug-resistant organisms among biofilm-forming bacteria further complicates treatment strategies. In tertiary care hospitals of developing countries such as India, catheter-associated urinary tract infections contribute significantly to nosocomial infections. Understanding the bacteriological profile, biofilm production, and antimicrobial susceptibility pattern of uropathogens is therefore essential for effective infection control and appropriate antibiotic selection. **Aim:** To determine the prevalence of biofilm production among uropathogens isolated from catheter-associated urinary tract infections and to evaluate their antimicrobial resistance pattern at Shadan Hospital, Hyderabad, Telangana. **Materials & Methods:** A hospital-based cross-sectional study was conducted in the Department of Microbiology, Shadan Hospital, Hyderabad, Telangana, over a period of one year. A total of 220 catheterized patients with symptoms suggestive of urinary tract infection were included in the study. Urine samples were collected aseptically from catheterized patients and cultured on Blood agar and MacConkey agar media. Identification of isolates was performed using standard microbiological techniques. Biofilm production was detected using the tube adherence method and the Congo red agar method. Antimicrobial susceptibility testing was performed by the Kirby–Bauer disc diffusion method according to CLSI guidelines. Statistical analysis was carried out using SPSS software. **Results:** Out of 220 urine samples analyzed, significant bacteriuria was observed in 168 (76.4%) samples, while 52 (23.6%) showed no bacterial growth. A total of 181 bacterial isolates were recovered, including polymicrobial growth in 13 samples. Escherichia coli was the predominant isolate, accounting for 32.0% of cases, followed by Klebsiella species (20.4%), Pseudomonas aeruginosa (14.4%), Enterococcus species (10.5%), Enterobacter species (9.9%), Citrobacter species (6.1%), Coagulase-negative Staphylococci (4.4%), and Staphylococcus aureus (2.2%). Among the isolates, 108 (59.7%) demonstrated biofilm production. Biofilm formation was highest among Pseudomonas aeruginosa (69.2%), followed by Klebsiella species (64.8%) and Escherichia coli (61.5%). Biofilm-producing isolates showed significantly higher resistance to fluoroquinolones, cephalosporins, and aminoglycosides compared to non-biofilm producers ($p<0.05$*). Carbapenems, nitrofurantoin, and piperacillin-tazobactam exhibited better sensitivity patterns against Gram-negative isolates. **Conclusion:** The present study demonstrates a high prevalence of biofilm-producing uropathogens among catheter-associated urinary tract infections in hospitalized patients. Biofilm-producing isolates exhibited increased antimicrobial resistance compared to non-biofilm producers, emphasizing the importance of early detection and appropriate antibiotic stewardship. Regular surveillance of antimicrobial resistance patterns and strict catheter care protocols are necessary to reduce the burden of CAUTIs and prevent the emergence of multidrug-resistant pathogens.

KEYWORDS : Biofilm, Uropathogens, Catheter-associated Urinary Tract Infection, Antibiotic Resistance, CAUTI, Antimicrobial Susceptibility.

INTRODUCTION

Urinary tract infection (UTI) is one of the most frequently encountered bacterial infections in clinical practice and represents a major cause of morbidity worldwide. Catheter-associated urinary tract infection (CAUTI) accounts for a substantial proportion of hospital-acquired infections and contributes significantly to increased healthcare burden, prolonged hospitalization, and antimicrobial resistance. Approximately 70–80% of nosocomial UTIs are associated with indwelling urinary catheters.[1]

Biofilm formation has emerged as an important virulence factor contributing to the persistence and chronicity of urinary tract infections. Biofilms are structured microbial communities enclosed within a self-produced extracellular polymeric matrix attached to biotic or abiotic surfaces.[2] The catheter surface provides an ideal environment for bacterial adhesion and colonization, ultimately resulting in biofilm development. Organisms embedded within biofilms exhibit enhanced resistance to antimicrobial agents and host immune responses, thereby increasing the likelihood of recurrent and persistent infections.[3]

The pathogenesis of CAUTI involves migration of microorganisms along the catheter surface, followed by adherence and biofilm maturation. Common uropathogens implicated in CAUTI include Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterococcus species, Proteus species, and Staphylococcal species.[4] Among these, Gram-negative bacilli predominate and frequently exhibit multidrug resistance patterns due to indiscriminate antibiotic use and prolonged hospitalization. [5]

Biofilm-producing bacteria are particularly difficult to eradicate because the extracellular matrix limits antibiotic penetration and facilitates horizontal transfer of resistance genes.[6] Furthermore, bacteria within biofilms demonstrate altered metabolic activity and reduced susceptibility to host defense mechanisms. Consequently, infections caused by biofilm-producing organisms are associated with treatment failure and recurrent infections.[7]

Several studies from India have reported increasing antimicrobial resistance among uropathogens isolated from catheterized patients.[8,9] However, regional variations exist in bacterial profile and antibiotic susceptibility patterns. Data regarding biofilm production and antimicrobial resistance among uropathogens in tertiary care hospitals of Hyderabad and Telangana remain limited.

Therefore, the present study was undertaken to evaluate the prevalence of biofilm formation among uropathogens isolated from catheter-associated urinary tract infections and to analyze their antibiotic resistance pattern at Shadan Hospital, Hyderabad, Telangana.

MATERIALS & METHODS

1. Study Design and Population: A hospital-based cross-sectional observational study was conducted in the Department of Microbiology at Shadan Hospital, Hyderabad, Telangana, over a period of one year. The study included catheterized patients admitted to various inpatient departments who developed symptoms suggestive of urinary tract infection after catheterization.

A total of 220 patients of both sexes and all age groups with indwelling

urinary catheters for more than 48 hours were included in the study. Patients with pre-existing urinary tract infection prior to catheterization and those receiving prolonged antibiotic therapy before catheterization were excluded from the study.

Institutional ethical committee approval was obtained before commencement of the study, and informed consent was taken from all participants or their attendants wherever applicable.

2. Group Allocation: The study population was categorized based on culture positivity and biofilm production. Isolates obtained from urine cultures were divided into biofilm-producing and non-biofilm-producing organisms according to the results of the Tube adherence test and Congo red agar method.

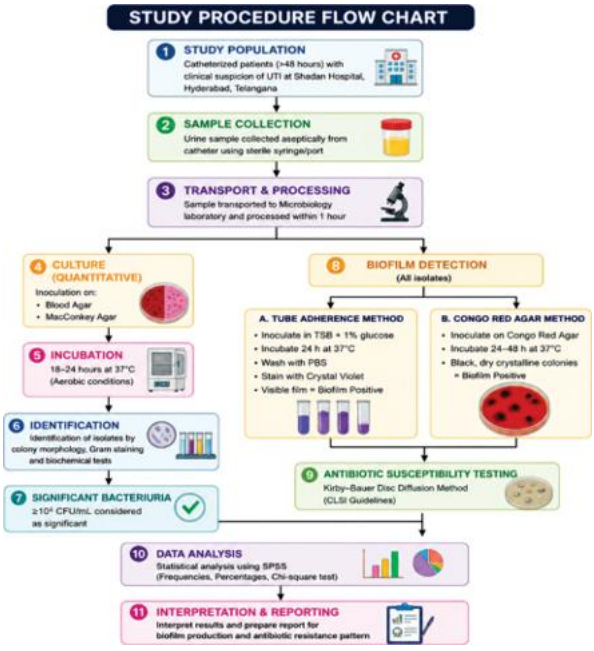
3. Study Procedure: Urine samples were collected aseptically from catheterized patients using sterile syringes after clamping the catheter tubing. Samples were transported immediately to the microbiology laboratory and processed without delay.

Urine specimens were inoculated on Blood agar and MacConkey agar plates using a calibrated loop technique and incubated aerobically at 37°C for 24 hours. Significant bacteriuria was defined as colony counts ≥105 CFU/mL.

Bacterial isolates were identified based on colony morphology, Gram staining characteristics, motility testing, and standard biochemical reactions. Biofilm production was detected by two methods:

i) **Tube Adherence Method:** Test organisms were inoculated into trypticase soy broth supplemented with 1% glucose and incubated at 37°C for 24 hours. The tubes were washed with phosphate-buffered saline, stained with crystal violet, and examined for visible film formation along the tube walls and bottom.

ii) **Congo Red Agar Method:** Isolates were inoculated on Congo red agar plates and incubated aerobically. Black colonies with dry crystalline morphology were interpreted as positive for biofilm production. Antimicrobial susceptibility testing was performed using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Antibiotics tested included Amikacin, Gentamicin, Ciprofloxacin, Levofloxacin, Ceftriaxone, Ceftazidime, Nitrofurantoin, Piperacillin-tazobactam, Imipenem, Meropenem, Doxycycline, and Cotrimoxazole.



4. Statistical Analysis: Data were entered into Microsoft Excel and analyzed using SPSS version 25.0. Categorical variables were expressed as frequencies and percentages. Chi-square test was used to compare antimicrobial resistance patterns between biofilm-producing and non-biofilm-producing isolates. A p-value <0.05 was considered statistically significant.

RESULTS

Out of 220 urine samples analyzed, significant bacteriuria was detected in 168 (76.4%) samples, whereas 52 (23.6%) samples showed no bacterial growth.

The incidence of CAUTI increased with prolonged duration of catheterization. Patients catheterized for more than 7 days demonstrated higher rates of significant bacteriuria compared to those catheterized for shorter durations. [Table & Figure - 1].

Among the culture-positive samples, polymicrobial growth was observed in 13 cases. A total of 181 bacterial isolates were recovered. [Table & Figure - 2].

Out of 181 isolates, 108 (59.7%) isolates demonstrated biofilm production, while 73 (40.3%) isolates were non-biofilm producers. [Table & Figure - 3].

The tube adherence method detected biofilm production in 92 isolates, whereas the Congo red agar method detected biofilm production in 81 isolates, indicating better sensitivity of the tube adherence method.

Biofilm-producing Gram-negative isolates exhibited higher resistance to ciprofloxacin (82.4%), ceftriaxone (76.8%), ceftazidime (71.3%), and gentamicin (68.5%). Better sensitivity was observed with imipenem (90.7%), meropenem (88.9%), nitrofurantoin (78.7%), and piperacillin-tazobactam (84.3%). [Table & Figure - 4]

A statistically significant association was observed between biofilm production and multidrug resistance among uropathogens ($p<0.05$*).

Table 1: Distribution of Significant Bacteriuria According to the Duration of Catheterization

Duration of Catheterization	Number of Patients (n=220)	Significant Bacteriuria n (%)
2 to 7 days	88	49 (55.7%)
8 to 14 days	102	94 (92.1%)
> 14 days	30	25 (83.3%)
Total	220	168 (76.4%)

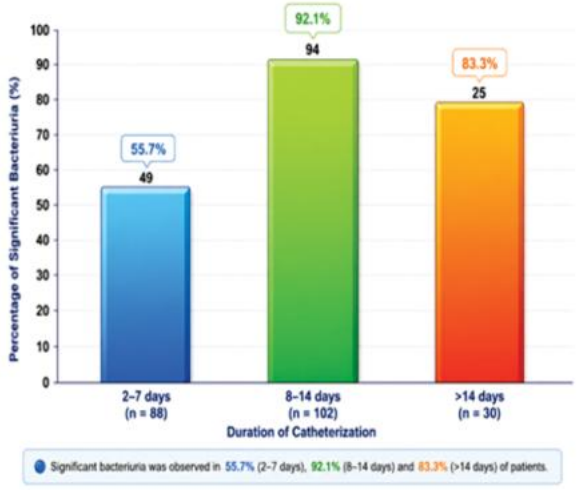


Figure 1: Distribution of Significant Bacteriuria According to the Duration of Catheterization

Table 2: Distribution of Uropathogens Isolated from Urine Samples

Organism	Number of Patients (n=220)	Percentage of Patients (%)
Escherichia coli	58	32%
Klebsiella species	37	20.4%
Pseudomonas aeruginosa	26	14.4%
Enterococcus species	19	10.5%
Enterobacter species	18	9.9%
Citrobacter species	11	6.1%
Coagulase-negative Staphylococci	08	4.4%
Staphylococcus aureus	04	2.2%
Total	181	100%

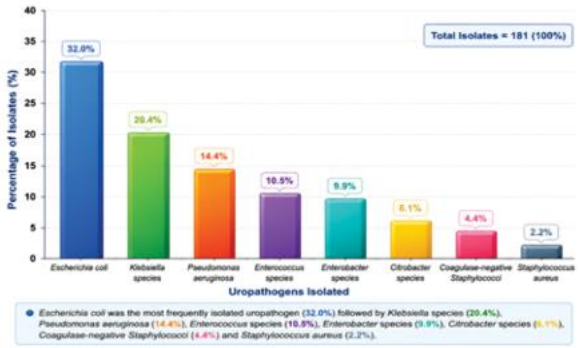


Figure 2: Distribution of Uropathogens Isolated from Urine Samples

Table 3: Biofilm Production Among Urinary Isolates

Organism (n)	Biofilm Positive n (%)	Biofilm Negative (n)
Pseudomonas aeruginosa (26)	18 (69.2%)	08
Klebsiella species (37)	24 (64.8%)	13
Escherichia coli (58)	36 (61.5%)	22
Enterobacter species (18)	10 (55.5%)	08
Enterococcus species (19)	9 (47.3%)	10
Citrobacter species (11)	5 (45.4%)	06
Staphylococcus aureus (4)	2 (50%)	02
CONS (8)	4 (50%)	04
Total	108 (59.7%)	73 (40.3%)

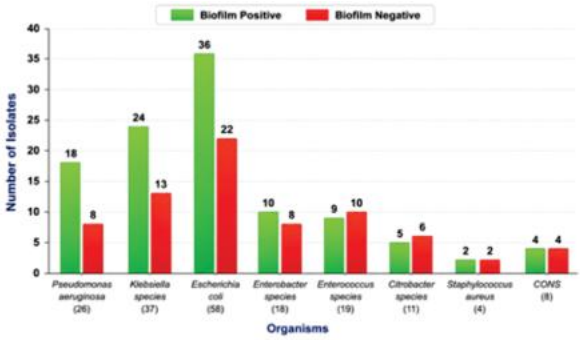


Figure 3: Distribution of Biofilm Producing and Non-Biofilm Producing Uropathogens Isolated from Catheter-Associated Urinary Tract Infections.

Table 4: Antimicrobial Susceptibility Pattern of Biofilm-Producing Gram-Negative Uropathogens

Antibiotic	Resistance	Sensitivity
Ciprofloxacin	82.4%	17.6%
Ceftriaxone	76.8%	23.2%
Ceftazidime	71.3%	28.7%
Gentamicin	68.5%	31.5%
Piperacillin–Tazobactam	15.7%	84.3%
Nitrofurantoin	21.3%	78.7%
Meropenem	11.1%	88.9%
Imipenem	9.3%	90.7%

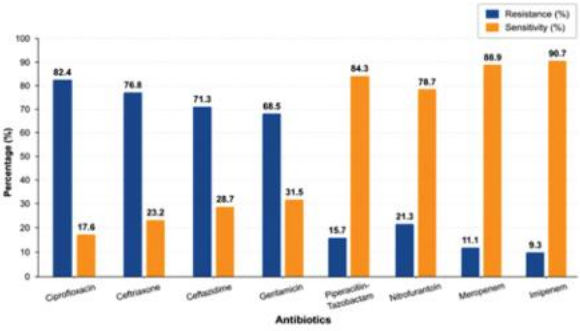


Figure 4: Antimicrobial Susceptibility Pattern of Biofilm-Producing Gram-Negative Uropathogens.

DISCUSSION

Catheter-associated urinary tract infection remains one of the most common healthcare-associated infections encountered in hospitalized patients.[10] The present study demonstrated a high prevalence of significant bacteriuria among catheterized patients, highlighting the importance of indwelling urinary catheters as a major risk factor for nosocomial infections.

In the current study, significant bacteriuria was observed in 76.4% of catheterized patients. Similar findings were reported by Patil et al.,[11] and Majumder et al.,[12] who observed culture positivity rates of 76% and 90%, respectively. The increased incidence of infection with prolonged catheterization observed in the present study is consistent with earlier reports emphasizing the role of catheter duration in bacterial colonization and biofilm formation.[13]

Escherichia coli was the predominant isolate in the present study accounting for 32.0% of isolates, followed by Klebsiella species and Pseudomonas aeruginosa. Similar bacteriological profiles have been documented in previous Indian and international studies.[14,15] The predominance of Gram-negative bacilli may be attributed to their enhanced virulence factors, including adhesins, fimbriae, and biofilm-forming ability.[16]

Biofilm formation was observed in 59.7% of isolates in the present study. Comparable findings were reported by Niveditha et al.,[17] who demonstrated biofilm production in 60% of uropathogens isolated from catheterized patients. Biofilm formation was highest among Pseudomonas aeruginosa and Klebsiella species, which correlates with their intrinsic capacity for surface adherence and extracellular polymeric matrix production.[18]

The tube adherence method demonstrated greater sensitivity in detecting biofilm formation compared to Congo red agar method. Similar observations were made by Hassan et al.,[19] who concluded that tube adherence method is a reliable and cost-effective screening technique for routine laboratory use.

An important observation in the present study was the significantly higher antimicrobial resistance among biofilm-producing isolates. Biofilm producers exhibited increased resistance to fluoroquinolones, cephalosporins, and aminoglycosides compared to non-biofilm-producing organisms. Similar findings have been reported by Pramodhini et al.,[20] who demonstrated a strong association between biofilm formation and multidrug resistance.

The reduced susceptibility of biofilm-producing organisms can be attributed to restricted antibiotic penetration, altered metabolic activity, and expression of resistance genes within the biofilm matrix.[21] Carbapenems, nitrofurantoin, and piperacillin-tazobactam exhibited better efficacy against Gram-negative isolates in the present study, which is comparable to findings reported in previous Indian studies.[12,20]

The emergence of multidrug-resistant biofilm-producing uropathogens poses a major challenge in clinical management. Continuous surveillance of antimicrobial resistance patterns and implementation of strict infection control practices are therefore essential to reduce the burden of CAUTI and prevent therapeutic failure.

CONCLUSION

The present study demonstrated a high prevalence of biofilm-producing uropathogens among catheter-associated urinary tract infections at Shadan Hospital, Hyderabad. Escherichia coli, Klebsiella species, and Pseudomonas aeruginosa were the predominant isolates, with a substantial proportion exhibiting biofilm formation and multidrug resistance. Biofilm-producing isolates showed significantly greater resistance to commonly used antibiotics compared to non-biofilm-producing organisms. Early detection of biofilm formation, rational antibiotic usage, periodic surveillance of resistance patterns, and strict catheter care practices are essential to minimize CAUTI-associated morbidity and prevent the emergence of multidrug-resistant pathogens.

Study Limitations

1. The study was conducted at a single tertiary care center, limiting the generalizability of the findings.
2. A relatively small sample size was included.
3. Biofilm detection was performed using phenotypic methods only; molecular confirmation was not carried out.

4. Molecular characterization of antimicrobial resistance mechanisms was not evaluated.
5. Patient-related risk factors and clinical outcomes were not analyzed in detail.
6. The study assessed only catheter-associated urinary tract infections and did not include non-catheterized UTI cases.
7. Long-term follow-up of patients was not performed to assess recurrence and treatment outcomes.

Future Scope

1. Multicentric studies with larger sample sizes can be conducted to obtain a broader understanding of biofilm-producing uropathogens across different populations.
2. Molecular characterization of biofilm-associated and antimicrobial resistance genes can be performed to better understand resistance mechanisms.
3. Quantitative methods for biofilm detection may be incorporated to assess the extent of biofilm formation.
4. Longitudinal studies can evaluate the impact of biofilm production on treatment outcomes, recurrence, and patient prognosis.
5. Studies assessing the effectiveness of novel anti-biofilm agents and preventive strategies against catheter-associated urinary tract infections are warranted.
6. Regular surveillance studies may help formulate region-specific antibiotic policies and infection control measures.

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